

SPHERICAL, MAGNETIC SiO_2 PARTICLES WITH AN ADJUSTABLE PARTICLE AND
PORE SIZE AND AN ADJUSTABLE MAGNETIC CONTENT, METHOD FOR PRODUCING
THEM, AND USE OF SiO_2 PARTICLES OF THIS TYPE

[Sphärische, magnetische SiO_2 -Partikel mit einstellbarer Teilchen un
Porengrösse sowie einstellbarem Magnetgehalt, Verfahren zu deren
Herstellung und Verwendung derartiger SiO_2 -Partikel]

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FOREIGN TITLE	[54A]:	SPHÄRISCHE, MAGNETISCHE SIO ₂ - PARTIKEL MIT EINSTELLBARER TEILCHEN UN PORENGRÖSSE SOWIE EINSTELLBAREM MAGNETGEHALT, VERFAHREN ZU DEREN HERSTELLUNG UND VERWENDUNG DERARTIGER SIO ₂ - PARTIKEL

Spherical, magnetic SiO₂ particles with an adjustable particle and pore size and an adjustable magnetic content, method for producing them, and use of SiO₂ particles of this type

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The present invention relates to magnetic SiO₂ particles with adjustable pore and particle size and adjustable magnetic content, a process for their production, and their use. Such SiO₂ particles are used, in particular, in the area of purification and analysis, in particular of biomolecules, and as carriers for biocatalysts, for example in the field of biotechnology, as adsorbers, for example in the field of medicine, or as a stimulus-response gel.

SiO₂ gels of various origin have long been known as chromatography media, although their use for nucleic acid purification was not known until the 80's and is described in DE 32 11 309 A1. The particles indicated there are pure SiO₂ gels, which are used exclusively in acid chromatography.

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Silanized iron-oxide particles for immobilizing enzymes are known from US 4,152,210 A. Also described for purposes of enzyme immobilization are the ferromagnetic particles in US 4,343,901, which are prepared by a sol-gel technique.

50-150 nm monodisperse magnetic particles are described in PCT EP97/04828, which comprise an SiO₂ core that is given its magnetic properties by coating it with iron oxide. By subsequent silanization

* Numbers in the margin indicate pagination in the foreign text.

of the iron oxide layer, the particles are made capable of binding nucleic acids.

Magnetic SiO_2 hybrid particles, consisting of a polystyrene core, onto which magnetite and then an SiO_2 layer are polymerized, are known from PCT/US 95/12988. These particles are used for antibody and cell separation.

US 4,280,918 A discloses iron oxide particles coated with colloidal SiO_2 .

Goetz et al. (Biotechn. & Bioengineering, Vol. 37, 614, 1991) describe 20-100 μm magnetic SiO_2 gel particles enabled for enzyme immobilization, which are produced by electrostatic coating of nickel powder with SiO_2 sols. Similarly, Homola and Lorenz (IEEE Transaction on Magnetism, Vol. 22, 716, 1986) describe an electrostatic coating of SiO_2 particles on Fe_2O_3 particles for producing magnetic storage media.

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Colloidal metal particles for use in immunoassay are described in application PCT/US97/03886.

0.2-3 μm magnetic particles are known from US 5,320,944 A, which are given magnetic properties by coating a polymer particle with iron oxide. By further coating the particles with silanes, nylon, or polystyrene, antibodies for use in immunoassay can then be coupled to the particles. WO 99/13993 describes the production of an optical sensor in the form of glass or transparent polymer carriers, on which

noble metal colloids are applied for improving effector molecule binding for use in immunoassay.

The synthesis of silica gels for gel-permeation chromatography, which are grafted with vinyl monomers by cerium(IV) initiation in order to suppress non-specific protein adsorption, is known from PCT/EP94/01378.

Organosilanized colloidal SiO₂ gel particles as biological separation media are disclosed in US 4,927,749 A and US 4,927,750 A and in WO 99/36359, whereby the stability of colloids and the type of silanization are emphasized; magnetic particles that contain a magnetic core material and are coated with an inorganic oxide are disclosed in EP 0 343 934 A1. Polymer particles that are coated with an additional polymer coating containing a magnetic material, on which a third polymer coating, which is enabled for interaction [translator's note: omission] -cules is applied, are described in /4 PCT/FR97/00912.

10-60 µm pearlescent pigments, which are sheathed with magnetite and are designed for separating biological mixtures, are found in PCT/DE97/01300.

Magnetic hybrid particles that comprise a polymer core that is first coated with a ferrofluid and then with a functional polyacrylate are subject matter of US Patent 5,648,124.

With regard to the separation of nucleic acids, particles known from the prior art have several disadvantages: first of all, a number

of carrier media are not magnetic (US 4,927,750 A, DE 32 11 309 A1, WO 99/36359 ; WO 94/26379), so that rapid separation of the particles, which is presently required for routine analysis, is not possible, i.e. column chromatographic methods are required, and secondly, magnetic particles based on SiO₂ or polystyrene coated with a magnetic oxide have a high specific density (WO 98/12717, US 4,152,210 A, EP 321 1309 A1, US 5,320,944 A). The result is an insufficient dispersibility, causing rapid degradation of the particles. This has a strong negative effect on the use of these particles in an immunoassay or nucleic acid assay, which is performed predominantly in suspension, so that additional mechanical mixing is required. However, the crucial disadvantage of the coated particles is that, despite the subsequent silanization, the metal oxides both as core material and as coating material, can come into direct /5 contact with the analytical solution. This presents a serious problem in nucleic acid analysis, e.g. in the polymerase chain reaction ("PCR"), since the polymerases used in the PCR can be activated when in contact with iron compounds. Moreover, for the magnetic particles known from the prior art, no process engineering measures are indicated that would lead to a controlled adjustment of the pore size - a parameter that has a crucial influence on the efficiency of nucleic acid purification.

The methods known from the prior art for producing magnetic particles are basically quite complex and the production process require hours.

Based on this prior art, it is the object of the present invention to make available magnetic SiO_2 particles, a method of producing them, and the use of said particles, whereby no labor-intensive and time-consuming coating techniques are required and where the particle size, pore size, and magnetic portion can be adjusted in a controlled manner.

This object is achieved by the method of producing magnetic SiO_2 particles in accordance with Claim 1, particles in accordance with Claim 68 or 69, the use of same in Claim 74. Advantageous refinements of the method of this invention of the particles in accordance with the invention are found in the respective dependent claims.

Starting point of the invention are prepolymers in the form of SiO_2 hydrosols, which are mixed with magnetic colloids or magnetic particles and are then polycondensed in heterogeneous phase to form spherical polymer particles. /6

Magnetic particles for the purposes of this invention are conventional magnetic particles, magnetic colloids, and/or ferrofluids, where by definition "colloids" and "ferrofluids" include all magnetic nanoparticles that form a stable aqueous colloidal

dispersion either with or without the addition of a stabilizer, surfactant, or emulsifier.

The SiO_2 sols are prepared using the known sol-gel methods by hydrolysis of alkoxysilanes with the help of dilute mineral acids or organic-based acids. Possible mineral acids include hydrochloric acid or carboxylic acids, such as acetic acid, formic acid, or propionic acid. To obtain the SiO_2 sols, the alkoxysilanes are dispersed in water and hydrolyzed by adding acid. Silicic acid orthoesters of aliphatic alcohols may be used as alkoxysilanes, methyl, ethyl, or propyl ester preferably being used alone or as mixtures. There is subsequently a condensation to lower polymeric SiO_2 hydrosols, which eventually by additional polycondensation result in more or less viscous sols and gels. This sol-gel process is generally carried out at lower temperatures, preferably ice-cooled. In order to better mix the heterogeneous silane-water phase, the reaction is carried out in an ultrasonic bath or with the help of an ultrasonic sonotrode. Depending on the composition, exposure to sonic waves lasts from 5 to 30 minutes, the times generally decreasing with increasing acid concentration. The mineral acids that are preferably used consistently have a concentration of 0.02 to 1 mol/liter, the volume fraction of the acids in the charge being 15-35%, preferably 20-28%. The monocarboxylic acids are used as pure acids, their volume fraction being 15-30%.

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The stoichiometry of the gel is determined by the type of hydrolysis and polycondensation. Thus, acid catalysis generally results in higher hydrolysis rates with decelerated polycondensation, while conversely the addition of bases promotes the polycondensation. As is generally known from the prior art, the specificity of the nucleic acid purification is critically determined by the pore structure, such that nucleic acid molecules having a molar mass > 50 kDa preferably are preferably separated on separation carriers with a pore size of 30 nm, while for nucleic acids < 50 kDa carriers with a pore size of 5-30 nm are particularly suitable.

Both reaction mechanisms resulting in a sol - hydrolysis and polycondensation - can be used to selectively alter or adjust the pore structure of the gels. Specifically, this can be achieved by producing the sol phase in the presence of a defined concentration of acid. Under otherwise identical test conditions, acid concentrations of > 0.15 mol/liter generally result in pore sizes of > 30 nm, while a reduction in the acid concentration to < 0.15 mol/liter is accompanied by a reduction in pore size to below 30 nm. The parameter adjustments mentioned are fundamentally linked to the respective conditions of the subsequent base-catalyzed polycondensation. /8

Thus, in general, the pore dimensions indicated above can consistently be produced by adding 1-3% ammonia solutions. On the other hand, the acid concentrations otherwise being the same, the use of more concentrated bases (6-25%) leads to pore diameters of

< 30 nm. In addition to the hydrolysis and polycondensation as structure-determining factors, it was surprisingly also found that with the use of both reaction mechanisms, the viscosity of the sol phase could be used to adjust the particle sizes.

The viscosity of the sols is derived directly from the specific type of reaction of the sol-gel formation during the aging process. During this process, water and alcohol are eliminated from the sol with the formation of oxo bridges, resulting in a parallel increase in viscosity. Other process conditions being equal (stirring speed, chemical composition), this rise in viscosity leads to an increase in particle size. Thus, in general particles with a size < 100 μm are formed primarily at a sol viscosity < 40 cp (or cP) and particles > 200 μm are formed from sols with a viscosity > 40 cp (or cP).

Due to the composition of the silane charge of this invention, the morphological requirements of the separation media are met, but surprisingly the requirements for the production of spherical magnetic SiO_2 particles are also created. These are:

a) miscibility of the sols with aqueous magnetic colloids or magnetic particle suspensions

b) formation of defined polymer drops upon dispersion in /9 suitable organic solvents.

As soon as the silane suspension has been homogenized by hydrolysis in the first production step, the sol can be used for

further particle production. However, immediate use of the sols is not necessarily required since, depending on the acid concentration and sol charge, the sols can be processed retain a fluid consistency over a period of days and can be processed even after a number of days.

In the second process step, a magnetic colloid or ferrofluid is mixed with the sol. By definition, "colloids" and "ferrofluids" include all magnetic nanoparticles that form a stable aqueous colloidal dispersion either with or without the addition of a stabilizer, surfactant, or emulsifier. Possible magnetic nanoparticles include magnetite and transition metal oxides, ferrite, or other nanoparticulate compounds of a ferromagnetic, ferrimagnetic, or superparamagnetic nature. Preparation of such colloids or ferrofluids has been described numerous times in: US 4,628,037; BR 1 439 031, EP 0275 285 A1, US 3,917,538 A ; US 4,827,945 A ; US 4,329,241 A.

The main criterion for selecting suitable colloids or ferrofluids is their compatibility with the SiO_2 sol, i.e. in contact with the sol phase, the colloid must not flocculate or agglomerate. Ferrofluids that enter into a homogeneous dispersion with the sols are those that contain either charged surfactants, e.g. in the form of aromatic or aliphatic sulfonic acid derivatives, or aliphatic carboxylic acids. Such fluids are also commercially available.

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Alternatively, magnetic colloids can also be produced without the addition of surfactants, emulsifiers, or other surface-active substances. For this purpose, the known precipitation methods from iron (II) and iron (III) salt solutions are used, as described for example by Khalafalla and Reimers (Br. Patent 143 9 03 1), Shinkai et al. (Biocatalysis, vol. 5, 61, 1991), or Kondo et al. (Appl. Microbiol. Biotechn., vol. 41, 99, 1994). Crucial to the synthesis of these colloids is the careful removal of the foreign ions, either by magnetophoresis or by dialysis. In magnetophoresis, the raw product is passed through a chromatographic column (diameter preferably 0.4-10 mm) tightly packed with steel wool, which is located between the poles of a strong permanent magnet or electromagnet. Due to the high-gradient magnetic field that is formed, the excess salt solution passes through the column unhindered, while the colloid or ferrofluid is retained. By repeated washing of the retained colloid, residual salts can largely be removed. After the column has been removed from the magnetic field or the field has been turned off, the colloid can then be retrieved by simple elution.

Apart from the use of the colloids and ferrofluids above, magnetic particles may also be used in principle for encapsulation that have a solid polymer covering, e.g. in the form of polyvinyl acetate, polyvinyl alcohol, dextran, polyacrolein, polystyrene, albumin, or alginate. Such magnetic particles, which typically

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have a particle size of 0.05 to 3 μm and are used in the field of biomolecule purification, are known from the prior art:

PCT/EP96/02398, J. Appl. Polymer Sci., vol. 50, 765, (1993), /Anal. Biochem., vol. 128, 342, 1983; US 4,654,267 A; J. Cell Sci., vol. 56, 157, 1982; Proc. Natl. Acad. Sci., vol. 78, 579, 1981, DE 35 08 000 A1; Magnetic particles of this type are also commercially available, e.g. under the names Dynabeads, BioMag, Estapor, M-PVA, AGOWA, BioBeads, or SPHERO, some of these names being registered trademarks.

These magnetic particles are used in a similar manner as the colloids and ferrofluids for producing the SiO_2 particles of this invention.

In the next step, the magnet-colloid-sol mixture is dispersed in an organic solvent with stirring. Solvents that are suitable as dispersing agents are those that are non-miscible with water and form either emulsions or dispersions. Surprisingly, it has been found that organic solvents, in particular, result in a stable dispersion, having a distribution coefficient > 2 . According to Laane et al. (in "Biocatalysis in Organic Media", Laane et al. Publ.: Elsevier, Amsterdam, pp. 65, 1987), the logarithm of the distribution coefficient is defined in a standard octanol-water two-phase system. Solvents that possess these properties are, for example, chloroform, trichloroethylene, 1,1,1-trichloroethane, hexane, petroleum ether, toluene, carbon tetrachloride, heptane, and octane. Mixtures of the

above-mentioned solvents having a density of ca. 1 g/cm³ are also suitable for dispersion.

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Basically, preparation of the magnetic particles is possible with the pure solvents indicated above. Surprisingly, for a narrower size distribution of the particles and for improved dispersion, it was shown that one or two emulsifiers or surfactants can be added to the organic phase. Additives of this type are, for example:

polyglycerin esters, polyethylene glycol-castor oil derivatives, polyoxyethylene-sorbitan fatty acid esters, alkylphenyl polyethylene glycol derivatives, block copolymers of castor oil derivatives, propylene oxide-ethylene oxide block copolymers, modified polyester, polyethylene glycol ether derivatives, polyoxypropylene-ethylene diamine block copolymers of sorbitan fatty acid esters, polyethylene glycols, polyoxyethylene and polyoxyethylene alcohol derivatives, and polyhydroxy fatty acid-polyethylene glycol block copolymers.

Substances of this type are commercially available, e.g. under the trade names: Hypermer®, Renex®, Estol®, Pluronic®, Eumulgin®, Tetronic®, Triton®, Brij®, Lameform®, Pripol®, Arlacel®, Prisorine®, Span®, Tween®, Dehymuls® or Synperonic®.

The emulsifier concentrations preferably used for the production of magnetic particles are between 0.1 and 15%, preferably between 0.5 and 6% by volume or weight.

In principle, of course, conventional vegetable oils and mixtures of vegetable oils and organic solvents can be used for

dispersion, but it has been shown that the use of organic solvents has several advantages over the oils. This is due to the low viscosity of the organic compounds, compared to that of the oils. /13

This low viscosity makes possible the separation of the synthesized magnetic particles from the reaction mixture within a few seconds, using a commercially available hand magnet. When vegetable oils are used, on the other hand, this separation step requires up to a few hours, followed by extensive washing processes with organic solvents, which are required for removing the residual oil from the magnetic particles. With the present process, on the other hand, the solvents that are used can easily be restored by redistillation. The volumetric ratios of organic phase to hydrosol are typically 8:1 to 30:1 and 2:1 to 4:1, in terms of the volumetric ratio of sol to magnetic colloid. The proportion by weight of the magnetic solid in the sol charge is between 10 and 55%. The possibility of simple and selective adjustment of the magnetic portion by simple admixture of the magnetic colloid, which distinguishes this process from the prior art, opens up a broad spectrum of applications that goes far beyond the simple separation of biomolecules and nucleic acids or biomolecule analysis, as described in W098/12712; DE 32 11 309 A1; US 5,320,944 A; US 4,927,749 A; US 4,927,750 A; WO 99/36359, PCT/FR97/00912. This relates, for example, to the use of magnetic particles as biocatalysts in biotechnological fermentation processes, as a protein-encapsuling matrix, as "stimulus-response" gels, or as

adsorbers in hemoperfusion. These additional applications result from the fact that, using the method of this invention and products, biomolecules such as enzymes or proteins can either be encapsulated in the sol-gel matrix by simple addition to the sol charges or covalently coupled to SiO_2 carriers via active groups, as will be described below.

In the last step, for fixing the magnetic SiO_2 dispersion to /14 defined magnetic particles a base is added during the mixing process. Within seconds, this addition of a base results in a solid gel formation of the polymer drop. The gel formation process is shorter, the higher the base concentration is chosen to be. Ammonia is preferably used as the base, although in principle other bases, such as NaOH, can also be used. The sodium hydroxide solution is generally used as a 0.05 to 0.1 molar solution and ammonia always in the form of a 1 to 12% aqueous solution. The volumetric ratios of base to sol are typically from 1:2 to 1:4.

Since the gelation reaction occurs very quickly, the production process requires less than an hour for the base particles, including synthesis of the sol and of the ferrofluid or colloid. This represents a time saving of 60 to 90%, compared to all known methods.

There are no particular limitations on mixing the sol-solvent charge - basically any common stirring devices can be used. For producing particularly fine particle fractions ($< 10 \mu\text{m}$), commercially

available dispersing tools are required that operate according to the rotor-stator principle (e.g. Ultra-Turrax®), with speeds of up to 20,000 rpm. There is an inverse proportionality between stirring speed and particle size, such that particle sizes between 20 and 500 nm [sic: μm ?] are generally produced by stirring speeds in the range of 800 to 1,800 rpm and particles between 0.5 and 10 μm require /15 stirring speeds of 5,000 to 20,000 rpm. The stirring process typically lasts 2 to 5 seconds. The magnetic particles that are produced can then be separated from the suspension using a hand magnet. This process is followed by a plurality of washings with alcohol and water. The magnet carriers that are produced are usually stored in water. The SiO_2 particles that are obtained can then be used directly for purifying nucleic acids or proteins, in accordance with the known methods.

In practice, not only carrier media based on pure SiO_2 have proven useful for the separation of nucleic acids, it is also known that media with cationic groups (anion exchangers), in particular, are highly suitable for nucleic acid separation. Carriers of this type are known from DE 32 11 309 and DE 37 17 209. This carrier type can be produced by chemical reaction of the SiO_2 particles with epoxy-substituted alkoxysilane, e.g. 3-glycidyloxypropyltrimethoxysilane or glycidyloxypropylmethyldiethoxysilane and subsequent nucleophilic opening of the oxiran ring with tertiary or secondary alkylamine. It

is also possible to synthesize strongly and weakly acidic ion exchangers and metal chelate carriers by reacting the epoxy-substituted SiO_2 particles described above, with the help of carboxylic acids, sulfites, thiosulfates, and amino-substituted carboxylic acids, e.g. nitrilotriacetic acid or iminodiacetic acid.

The chemical modification of the SiO_2 particles is not limited to the production of ion exchangers. In one particular embodiment, the SiO_2 carriers can be reacted with substituted alkylalkoxysilanes of the general formula $\text{X}-(\text{CH}_2)_n-\text{Si}-(\text{OR})_3$, where X is a halogen, cyano, /16 NH_2 - or mercapto radical, $n = 1-6$, preferably 3, R is an alkyl, trialkysilyl radical, or H. For the separation in accordance with the affinity principle or for use as biocatalysts, ligands in the form of peptides, proteins, or enzymes can be coupled covalently to the carriers that have been modified in this way. Several possibilities are available for this purpose:

1. Protein and other ligands can be directly coupled to the halogen-substituted carriers by simple incubation.
2. Protein ligands can be covalently bonded to the amino group-substituted magnetic particles using dialdehydes.
3. Amino-substituted SiO_2 carriers are first converted using succinic anhydride and the carboxyl groups that are formed are then activated, for example, with carbodiimides. Protein ligands can be directly coupled to the activated groups.

4. Binding of protein and other ligands by way of the oxiran groups of the epoxy-substituted SiO_2 particles described above.

As with the proteins, amino-functionalized nucleic acids of any kind can also be coupled to the SiO_2 carriers using the methods described above.

Without going into further detail on these couplings and modifications, which are described among other places in /17
"Scientific and Clinical Applications of Magnetic Carriers," Häfeli et al. (Eds.), Plenum Press, New York, 1997, and Shriver-Lake in "Immobilized Biomolecules in Analysis," T. Cass and F. S. Ligler Eds., Oxford University Press, 1998, it is assumed that those skilled in the art in this field is sufficiently familiar with the special reaction methods and, thus, can utilize the description in principle. Thus, the embodiments described are in no way seen as limiting disclosures.

In addition to the possibility of producing magnetic particles from pure SiO_2 gel, it was surprising found that the magnetic particles can also be produced by mixing SiO_2 sols with synthetic, semi-synthetic, or natural organic polymers. Considering the changed morphology and chemical reaction behavior compared to pure SiO_2 gels, a modified spectrum of properties is obtained that opens up additional applications. This is particularly true of applications in the area of purifying high-molecular nucleic acids and the separation

of proteins. With polymer blends it is possible to produce hybrid polymers that in part combine both the chemicophysical properties of organic polymers and those of the SiO_2 gels. In particular, the adsorption behavior of the hybrid particles with respect to proteins and the mechanical strength and alkali stability may be mentioned.

Possible copolymers are mainly those that are water-soluble and compatible with the SiO_2 sols, i.e. those that can form homogeneous mixtures without phase separation. With regard to the products in accordance with this invention and their selective application in certain biochemical and biotechnological areas, such polymers are preferred that make possible both a selective modification of the morphological structure with regard to an increase in surface and with regard to the pore structure. /18

Polymers that are preferred as additives are, for example, polyvinyl alcohol, polyacrylic acid, polyamino acids, polysaccharides, proteins, and polyvinyl pyrrolidone. In accordance with this invention, 0.5-5% aqueous polymer solutions are generally used that are mixed with the SiO_2 sols before the dispersion process. The volume fraction of the organic polymer solutions in the sol phase is between 5 and 25%. Polymers having a molar mass of 15,000 to 250,000 are generally used.

With regard to the separation of proteins, the use of predominantly highly hydrophilic polymers also offers the possibility

of largely suppressing the strong nonspecific adsorption behavior found with pure silica gels.

The addition of organic polymers to the sol charge basically changes nothing in the method described above for producing pure SiO_2 particles.

Some examples of the method in accordance with this invention and of SiO_2 particles will be described below.

Example 1

A mixture of 55 ml tetraethoxysilane and 15 ml 0.05 M HCl are subjected to ultrasound in an ultrasonic bath with ice cooling for /19 25 min. 20 ml of the SiO_2 sol, which has a viscosity of 36 cp at 20°C, is mixed with 5 ml magnetic colloid, which has been prepared in accordance with the specifications of Shinkai et al. (Biocatalysis, vol. 5, 61, 1991) by oxidation of a 0.6 molar iron (II) salt solution using 0.3 M Na nitrite. The suspension that is obtained added to 280 ml 1,1,1-trichloroethane, in which 0.5 vol% Tween 80 and 0.8 vol% Prisorine are dissolved. The charge is dispersed several seconds with stirring (1800 rpm), 10 ml 1% ammonia solution is then added, and the dispersion is stirred an additional 3 seconds. After 5 minutes the magnetic particles are separated from the suspension by a hand magnet and rewashed three times, in each case with ca. 50 ml ethanol and water. Magnetic particles having a particle size of 20-60 μm are obtained. After 12 hours of incubation in water, the particles are

again washed several times with water and then dried under a vacuum for several hours until their weight is constant. The particles have a mean pore size of 24 nm. The magnetic particles obtained can be used in accordance with the known methods for purifying nucleic acids.

Example 2

A mixture consisting of 25 ml of the sol produced in accordance with Example 1 and 8 ml magnetite colloid, which is prepared according to the instructions of Khalafalla and Reimers (Br Pat. 1 439 03 1) from an iron (III)/iron (II) salt solution (molar ratio 2:1) by ammonia precipitation, is dispersed in 300 ml toluene, in which in each case 2 vol% Pripol 1009 and Tween 20 are dissolved, /20 using an Ultra-Turrax at 12,000 rpm. After the addition of 12 ml 3% ammonia solution, it is dispersed an additional 2 seconds. Separation and processing in accordance with Example 1 follow. Magnetic particles having a particle size of 1-4 μm and a Magnetil content of 38 wt% are obtained.

Example 3

A suspension consisting of 50 ml tetramethoxysilane and 20 ml 0.3 M HCl are subjected to ultrasound for 5 min in an ultrasonic bath. 20 ml of the sol obtained in this way, which has a viscosity of 48 cP, is mixed with 8 ml of the magnetite colloid prepared as in Example 2 and 2 ml of a 2.5% polyvinyl alcohol solution (molar mass = 224,000). The suspension is then dispersed with stirring (1,600 rpm)

in 320 ml hexane, which contains 0.5 vol% Estol SMO 3685 dissolved in it. During the dispersion process, 10 ml 1% ammonia solution is added and the result is stirred an additional 4 seconds. Separation and processing of the magnetic particles thus obtained occur as in Example 1. Carriers are produced with a size of 80-150 μm and an average pore size of 58 nm. The separated magnetic particle fraction is washed 5 times with dried toluene after the respective magnetic separation and then refluxed for 12 hours after the addition of 50 ml toluene and 0.85 g 3-aminopropyltrethoxysilane. The magnetic particles are again separated magnetically and again washed 3 times, in each case with toluene and chloroform. The result is dried for several hours under a vacuum. The amino-modified product is then reacted with 6% glutaraldehyde solution in 25 ml 0.1 M Na carbonate /21 buffer, pH 9.2, for 3 hours at 35°C. It is then rewashed intensively with 0.1 M phosphate buffer, pH 7.2. The aldehyde-functionalized magnetic particles that are obtained are suspended in 15 ml 0.1 M phosphate buffer. 2 ml of the suspension is magnetically separated and incubated in 2 ml 0.1 M phosphate buffer, pH 7.2, in which 1.2 mg streptavidin is dissolved. After a 6-hour reaction at 35°C, the product is rewashed 5 times with phosphate buffer. In order to saturate any remaining residual aldehyde groups, the magnetically separated product is incubated in 5 ml 0.2 M ethanolamine at room temperature for 5 hours. The magnetic particles, which are then washed several times with phosphate buffer, can be used directly by

known methods for binding biotinylated nucleic acids or biotinylated proteins.

Claims

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1. A method for producing magnetic SiO₂ particles, wherein

a) alkoxysilanes are dispersed in water, acid-catalytically hydrolyzed, and condensed to form an SiO₂ hydrosol,

b) magnetic particles, for example conventional magnetic particles, magnetic colloids, and/or ferrofluids, are admixed with the SiO₂ hydrosol to produce a magnetic particle sol mixture,

c) the magnetic particle sol mixture is dispersed in an organic solvent that is immiscible with water, and

d) a base is added to the magnetic particle sol mixture during or after the dispersion in the organic solvents for gel formation.

2. A method as recited in the previous Claim, characterized in that vegetable oils or organic solvents having a distribution coefficient greater than 2 or mixtures thereof are used as the solvent.

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3. A method as recited in one of the previous Claims, characterized in that chloroform, trichloroethylene, 1,1,1-trichloroethane, hexane, petroleum ether, toluene, carbon tetrachloride, heptane, octane, or mixtures thereof are used as the solvent.

4. A method as recited in one of the previous claims, characterized in that the viscosity of the SiO₂ hydrosol is adjusted in such a way that the SiO₂ particles that are produced have a predetermined diameter.

5. A method as recited in one of the previous claims, characterized in that the viscosity of the SiO₂ hydrosols is adjusted to between 5 cp and 500 cp.

6. A method as recited in Claim 4 or 5, characterized in that, for producing SiO₂ particles having a diameter of less than 100 μm, the viscosity of the sol is adjusted to less than 40 cp.

7. A method as recited in Claim 4 or 5, characterized in that, for producing SiO₂ particles having a diameter of greater than /24 200 μm, the viscosity of the sol is adjusted to greater than 40 cp.

8. A method as recited in one of the previous claims, characterized in that the dispersion of hydrosol, magnetic particles, organic solvent, and base is stirred mechanically.

9. A method as recited in the previous Claim, characterized in that the dispersion is stirred for ca. 2 to 5 seconds.

10. A method as recited in one of the two previous Claims, characterized in that the dispersion is stirred at such a speed that a predetermined diameter of the SiO₂ particles is achieved.

11. A method in accordance with the previous Claim, /25 characterized in that the dispersion is stirred at 800-1,800 rpm.

12. A method as recited in Claim 51, characterized in that the dispersion is stirred at 5,000 to 20,000 rpm.

13. A method as recited in one of the previous claims, characterized in that silicic acid orthoesters of aliphatic alcohols are used as the alkoxysilanes.

14. A method as recited in Claim 13, characterized in that methyl, ethyl, and/or propylester is used as the ester.

15. A method as recited in one of the previous claims, characterized in that diluted acids and/or organic-based acids are added to the alkoxysilanes for acid-catalytic hydrolysis.

16. A method as recited in one of the previous claims, /26
characterized in that hydrochloric acid, formic acid, or propionic acid is added to the alkoxysilanes for acid-catalytic hydrolysis.

17. A method as recited in one of the previous claims, characterized in that mineral acids having a concentration between 0.02 and 1 mol/liter are added for acid-catalytic hydrolysis.

18. A method as recited in one of the previous claims, characterized in that mineral acids at a volume fraction of 15 to 35% in the dispersion are added for acid-catalytic hydrolysis.

19. A method as recited in one of the previous claims, characterized in that mineral acids at a volume fraction of 20 to 28% are added to the dispersion for acid-catalytic hydrolysis.

20. A method as recited in one of the previous claims, characterized in that monocarboxylic acids as pure acids are added for acid-catalytic hydrolysis.

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21. A method as recited in one of the previous claims, characterized in that monocarboxylic acids at a volume fraction of 15 to 30% in the dispersion are added for acid-catalytic hydrolysis.

22. A method as recited in one of the previous claims, characterized in that the acid concentration in the dispersion is adjusted in such a way that the SiO_2 particles that are produced have a predetermined pore size.

23. A method as recited in the previous Claim, characterized in that for producing SiO_2 particles with a pore size greater than ca. 30 nm the acid concentration is adjusted to greater than 0.15 mol/liter.

24. A method as recited in Claim 22, characterized in that for producing SiO_2 particles with a pore size smaller than ca. 30 nm the acid concentration is adjusted to less than 0.15 mol/liter.

25. A method as recited in one of the previous claims, characterized in that the condensation of the alkoxysilanes in step a) is carried out at low temperatures.

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26. A method as recited in the previous Claim, characterized in that the condensation of the alkoxysilanes in step a) is carried out with ice cooling.

27. A method as recited in one of the previous claims, characterized in that the aqueous dispersion of alkoxysilanes is subjected to ultrasound for at least a time during step a).

28. A method as recited in one of the previous claims, characterized in that the aqueous dispersion of alkoxysilanes is subjected to ultrasound for ca. 5 to 30 minutes during step a).

29. A method as recited in one of the previous claims, characterized in that, for producing a magnetic particle sol mixture, magnetic particles in the form of magnetic colloids, in particular colloids of magnetic nanoparticles, or ferrofluids, in particular fluids with magnetic nanoparticles, and/or magnetic nanoparticles in general, are added to the SiO₂ hydrosol. /29

30. A method as recited in the previous Claim, characterized in that magnetite, transition metal oxides, ferrite, and/or other nanoparticulate, ferromagnetic, ferrimagnetic, or superparamagnetic compounds are admixed as magnetic nanoparticles.

31. A method as recited in one of the previous claims, characterized in that stabilizers, surfactants, and/or emulsifiers are added to magnetic particles for forming a stable aqueous colloidal dispersion of the magnetic particles in the SiO₂ hydrosol.

32. A method as recited in one of the previous claims, characterized in that, as magnetic particles, ferrofluids are added that form a homogeneous dispersion with the SiO₂ hydrosol.

33. A method as recited in one of the previous claims,

characterized in that ferrofluids that contain charged surfactants, /30
aromatic or aliphatic sulfonic acid derivative, and/or aliphatic
carboxylic acids are admixed as magnetic particles.

34. A method as recited in one of the previous claims,
characterized in that magnetic particles are added that have a solid
and/or insoluble polymer covering.

35. A method as recited in the previous Claim, characterized in
that magnetic particles are added that have a polymer coating
containing or comprising polyvinyl acetate, polyvinyl alcohol,
dextran, polyacrolein, polystyrene, albumin, and/or alginate.

36. A method as recited in one of the previous claims,
characterized in that the magnetic particles have a diameter between
0.05 μm and 3 μm .

37. A method as recited in one of the previous claims,
characterized in that the magnetic particles are added in such a way
that the volumetric ratio of hydrosol to magnetic particles is /31
between 2:1 and 4:1 and/or the proportion by weight of magnetic
particles in the magnetic colloid-sol mixture is between 10% and 55%.

38. A method as recited in one of the previous claims,
characterized in that the magnetic particle-sol mixture is dispersed
for 1 to 5 seconds in an organic solvent that is immiscible with
water.

39. A method as recited in one of the previous claims,
characterized in that the magnetic particle-sol mixture is dispersed

in an organic solvent that is not miscible with water and that forms emulsions or dispersions.

40. A method as recited in one of the previous claims, characterized in that surface-active substances, emulsifiers, and/or surfactants are added to the solvent.

41. A method as recited in the previous Claim, characterized in that polyglycerin ester, polyethylene glycol-castor oil derivatives, polyoxyethylene sorbitan fatty acid esters, alkyl phenyl /32
polyethylene glycol derivatives, block copolymers of castor oil derivatives, propylene oxide-ethylene oxide block copolymers, modified polyester, polyethylene glycol-ether derivatives, polyoxypropylene ethylene diamine block copolymers, sorbitan-fatty acid esters, polyethylene glycols, polyoxyethylene derivatives, polyoxyethylene-alcohol derivatives, or polyhydroxy fatty acid-polyethylene glycol block copolymers are added to the solvent as surface-active substances.

42. A method as recited in one of the two previous Claims, characterized in that the portion of surface-active substances, emulsifiers, and/or surfactants in the organic solvent is between 0.1 vol% and 25 vol%.

43. A method as recited in one of the three previous Claims, characterized in that the surface-active substances, emulsifiers, and/or surfactants are added at a concentration in the dispersion of between 0.1 and 15 vol% or wt%.

44. A method as recited in the previous Claim, characterized in that the surface-active substances, emulsifiers and/or surfactants are added at a concentration in the dispersion of between 0.5 and 6 vol% or wt%. /33

45. A method as recited in one of the previous claims, characterized in that the solvent is added in such a way that the volumetric ratio of solvent to hydrosol is between 5:1 and 30:1, preferably between 8:1 and 30:1.

46. A method as recited in one of the previous claims, characterized in that the base in process step d) is added at a concentration such that the SiO_2 particles that are produced have a predetermined pore size.

47. A method as recited in the previous Claim, characterized in that, for producing SiO_2 particles with a pore size greater than ca. 30 nm, the final concentration of the base that is added is between 1 and 3%. /34

48. A method as recited in Claim 46, characterized in that, for producing SiO_2 particles with a pore size smaller than ca. 30 nm, the final concentration of the base that is added is between 6 and 25%.

49. A method as recited in one of the previous claims, characterized in that ammonia is used as the base.

50. A method as recited in the previous Claim, characterized in that the ammonia is used in the form of a 1% to 12% aqueous solution..

51. A method as recited in one of the previous claims, characterized in that sodium hydroxide solution (NaOH) is used as the base.

52. A method as recited in the previous Claim, characterized in /35 that the sodium hydroxide solution is used in the form of a 0.05 to 0.1 molar solution.

53. A method as recited in one of the previous claims, characterized in that the volumetric ratios of the base and hydrosol added are between 1:2 and 1:4.

54. A method as recited in one of the previous claims, characterized in that the magnetic SiO₂ particles are removed from the dispersion, optionally with a hand magnet, and optionally purified.

55. A method as recited in the previous Claim, characterized in that the magnetic SiO₂ particles removed from the dispersion are washed one or more times with alcohol and/or water.

56. A method as recited in one of the two previous Claims, characterized in that the SiO₂ particles removed from the dispersion and optionally purified are stored in an aqueous medium. /36

57. A method as recited in one of the previous claims, characterized in that the SiO₂ particles are reacted with alkoxysilanes and/or substituted alkoxysilanes, epoxy-substituted alkoxysilane, 3-glycidyloxypropyl trimethoxysilane, and/or 3-glycidyloxypropylmethyl diethoxysilane, and then subjected to a

nucleophilic opening of the oxiran ring by means of tertiary or secondary alkyl amines.

58. A method as recited in the previous Claim, characterized in that the SiO_2 particles are reacted with epoxy-substituted alkoxy silane, 3-glycidyloxypropyl trimethoxysilane or 3-glycidyloxypropylmethyl diethoxysilane, and then with carboxylic acids, sulfites, thiosulfates, or amino substituted carboxylic acids, nitrilotriacetic acid, or iminodiacetic acid.

59. A method as recited in one of the previous claims, characterized in that the SiO_2 particles are reacted with substituted alkylalkoxysilanes of the general formula $\text{X}-(\text{CH}_2)_n-\text{Si}-(\text{OR})_3$, where X is a halogen, cyano, NH_2 - or mercapto radical, $n = 1$ to 6, preferably 3, R is an alkyl or trialkysilyl radical, or H. /37

60. A method as recited in the previous Claim, characterized in that ligands, e.g. peptides, proteins, and/or enzymes, are subsequently covalently coupled to the SiO_2 particles.

61. A method as recited in one of the previous claims, characterized in that synthetic, semi-synthetic, and/or natural organic polymers can be admixed with the SiO_2 hydrosol.

62. A method as recited in the previous Claim, characterized in that water-soluble polymers and/or polymers that form homogeneous mixtures with the SiO_2 hydrosol without phase separation are used as organic polymers.

63. A method as recited in one of the two previous Claims, characterized in that organic polymers having a molar mass of between 15,000 and 250,000 Da are added.

64. A method as recited in one of the three previous Claims, characterized in that polysaccharides, proteins, polyvinyl alcohol, /38 dextran, starch, chitosan, alginate, Ficoll, polyethyleneimine, agarose, polyacrylic acid, polyamino acid, polyvinyl pyrrolidone, hyaluronic acid, and/or pectinate are used as organic polymers.

65. A method as recited in one of the Claims 61 through 64, characterized in that the organic polymers are added as a solution.

66. A method as recited in one of the Claims 61 through 65, characterized in that the organic polymers are added as 0.5% to 5% solutions.

67. A method as recited in one of the Claims 61 through 66, characterized in that the organic polymers are added in such a way that the portion of polymer solution in the hydrosol is between 1 and 30 vol%, advantageously between 5 and 25 vol%.

68. Magnetic SiO₂ particles, obtainable by a method as recited in one of the previous Claims.

69. Magnetic SiO₂ particles, optionally in accordance with the /39 previous Claim, having a particle diameter of 0.5 μm to 2,000 μm, characterized in that, in the SiO₂ particles, magnetic particles, for example as a magnetic colloid or ferrofluid, are homogeneously

distributed over the entire SiO₂ particle and encapsulated, the portion of magnetic particles in the entire weight of the SiO₂ particle being between 10 and 55%.

70. Particles as recited in one of the two previous Claims, characterized in that they contain between 2 and 25 wt% organic polymers, polysaccharides, and/or proteins.

71. Particles as recited in one of the three previous Claims, characterized in that the surface of the SiO₂ particles has groups that can be coupled with oligopeptides, oligosaccharides, oligonucleotides, polypeptides, polynucleotides, polysaccharides, antibodies, antibody fragments, and/or enzymes.

72. Particles as recited in one of the Claims 68 through 71, characterized in that the surface of the SiO₂ particles is epoxy-substituted.

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73. Particles as recited in the previous Claim, characterized in that the epoxy-substituted SiO₂ particles are reacted with secondary or tertiary alkyl amines to form anion exchangers.

74. Use of magnetic particles as recited in one of the Claims 68 through 73 for purification or analysis of nucleic acids, proteins, peptides, antibodies, or antibody fragments, or other biomolecules or biotinylated derivatives thereof, as ion exchangers or metal-chelate carriers, as carriers for biocatalysts such as peptides, proteins, and/or enzymes, as biocatalysts in biotechnological fermentation

processes, as a protein-encapsulation matrix, as a "stimulus-response" gel, or as an adsorber, e.g. in hemoperfusions.